Chinese Chemical Letters Vol. 16, No. 10, pp 1357-1360, 2005
http://www.imm.ac.cn/journal/ccl.html

Glucose Biosensor Based on Carbon/PVC-COOH/Ferrocene Composite with Covalently Immobilized Enzyme

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Abstract: A carbon/PVC-COOH/ferrocene composite electrode used for the determination of glucose has been prepared. The ferrocene acted as mediator was incorporated into the PVC-COOH polymer and the leakage could be prevented. The presence of carboxyl groups on the electrode surface allowed immobilizing enzyme *via* EDC and NHS. The ratio of PVC-COOH to graphite powder (w/w) has been studied. Amperometric determination of glucose has been performed at potential of 0.30 V *vs* SCE. The response time was < 15 s. The linear response range was of 0.1-20 mmol/L with a detection limit of 48 μmol/L.

Keywords: PVC-COOH, ferrocene, glucose oxidase, glucose.

Poly(vinyl chloride)(PVC), a high molecular plasticized polymer has been used extensively for the fabrication of ion-selective electrodes (ISEs) [1-3]. The electrodes using PVC to incorporate porphyrin, porphrins metal complexes, or neutral carriers have been reported to show an excellent sensitivity and long-term stability. The carboxylic poly(vinyl chloride)(PVC-COOH) was also used as an immobilization matrix to immobilize urease for the fabrication of a renewable urea sensor [4]. In this work, a simple glucose biosensor using PVC-COOH as an immobilization matrix was prepared. The sensor was finished by filling the mixture of PVC-COOH, ferrocene and graphite powder into a teflon tube. This procedure could prevent the leakage of ferrocene. The carboxyl groups on the electrode surface were used to immobilize glucose oxidase *via* EDC and NHS [5-6]. The results of the analyzed real samples were satisfactory.

Experimental

Glucose oxidase (GOD, from *Aspergillus niger*; EC 1.1.3.4, 196,000 U g^{-1}), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxylsuccinimide (NHS) were purchased from Sigma. Carboxylated poly (vinyl chloride) (PVC-COOH) and ferrocene were obtained from Aldrich. β-D-Glucose was from ICN. All other reagents and solvents were of analytical grade, and doubly distilled water was used throughout.

Cyclic voltammetric and amperometric experiments were carried out with PAR 273

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potentiostat/galvanostat and model 270 software (EG&Gprinceton Applied Research, Princeton, NJ, USA) and Model XJP-821 polargraphic analyzer (Jiangsu Electroanalytical Instruments, Jiangsu, China). A three-electrode system consisted of a glucose biosensor as the working electrode, a saturated calomel electrode (SCE) as the reference and a platinum foil as the auxiliary electrode. Electrochemical measurements were performed in an electrochemical cell holding 10 mL of 0.1 mol/L PBS solution.

The carbon/PVC-COOH/ferrocene composite electrode was prepared as follows: 100 mg of PVC-COOH and 50 mg of ferrocene were dissolved in 2 mL of THF. After being stirred thoroughly, 600 mg of graphite powder was added into the solution and mixed. When THF evaporated, 400 mg of paraffin melted at 60°C was added into the mixture and blended thoroughly to obtain a homogeneous paste. Finally, the mixture was filled into a teflon tube and pressed tightly. A copper wire was inserted through the opposite end to establish an electric contact. The composite electrode was polished with ultra fine emery paper. The ferrocene-doped carbon paste electrode without PVC-COOH was prepared by the same method except that only ferrocene was dissolved in THF.

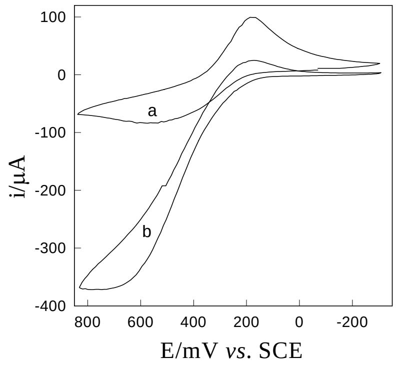
The composite electrode was initially activated by soaking in an aqueous solution of EDC (100 mg/mL) and NHS (100 mg/mL) for 1.5 h. After being rinsed with water, 20 μL of enzyme solution (20 mg/mL) was coated on the surface of electrode. 1.5 h later, the electrode was washed to remove the excess of unbound enzyme. Then, it was kept under dry conditions at 4°C before use.

Results and Discussion

Figure 1a shows cyclic voltammogram (CV) of the carbon/PVC-COOH/ferrocene composite/enzyme electrode in PBS (pH 7.0) without glucose. There was a pair of *quasi*-reversible anodic and cathodic waves, which indicated the ferrocene immobilized in the PVC-COOH polymer had enough flexibility and could transport electrons freely in the matrix. When glucose was added, the obvious electrocatalytic characteristics appeared along with great increase of the oxidation current and decrease of reduction current (**Figure 1b**). These phenomena indicated that the immobilized ferrocene shuttled electrons effectively from the electrode surface to the active center of GOD.

The electrochemical performance of the biosensor was greatly affected by the ratio of PVC-COOH to graphite powder (w/w) as PVC-COOH provided the carboxyl groups to immobilize GOD. When the ratio was small (**Figure 2a**), the amount of carboxyl groups on the electrode surface was also very small. Thus, the amount of glucose oxidase immobilized onto the electrode surface would be limited and the biosensor would exhibit low sensitivity and narrow dynamic linear range. As the ratio increased (**Figure 2b-d**), both the sensitivity and linear span increased. Further increase of the ratio did not very benefit the current response (**Figure 2e**). As PVC-COOH was a non-conductive material, over amount of PVC-COOH would increase the background current of response signal and affect the detection limit of the biosensor. In addition, when the ratio was too high, the electrode would become fragile and crack easily. So an optimum ratio of PVC-COOH to graphite powder was important for a good glucose biosensor. Here, 1/6 was chosen.

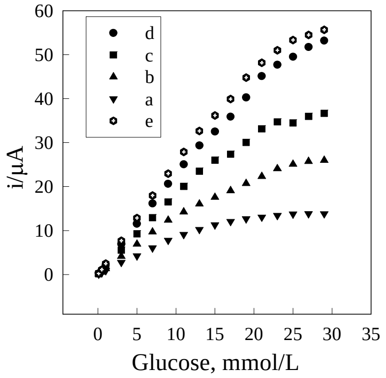
Figure 1 Cyclic voltammograms of the biosensor at a scan rate of 100 mV s^{-1}



(a) Without and (b) with 25 mmol/L glucose in 0.1 mol/L PBS (pH 7.0).

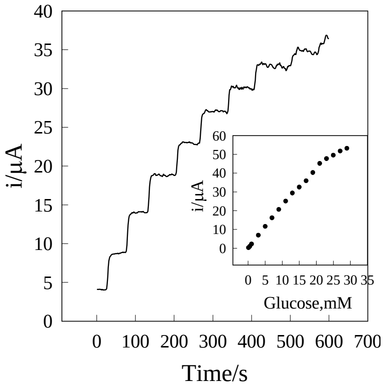
Both of the carbon/PVC-COOH/ferrocene composite electrode and the ferrocene-doped carbon paste electrode without PVC-COOH were soaked in phosphate buffer (pH 7.0) under vigorous stirring for 12 h. For the carbon paste electrode, the peak current decreased to only 44.6% of its initial value. But, at the composite electrode, the peak current remained 95% of its initial value. It implied that the ferrocene in the composite matrix was very stable and the PVC-COOH polymer prevented the leakage of ferrocene.

Figure 2 Effect of the ratio of PVC-COOH /graphite powder (w/w) in the composite electrode on the response of glucose biosensor



The ratio of PVC-COOH/graphite powder (w/w): (a) 1/15; (b) 1/10; (c) 2/15; (d) 1/6. (e) 1/5. Supporting electrolyte: 0.1 mol/L PBS (pH 7.0); operating potential: 300 mV *vs.* SCE.

Figure 3 Typical current-time response for successive addition of 2 mmol/L glucose in 0.1 mol/L PBS (pH 7.0)



Operating potential: 300 mV *vs.* SCE. The insert shows a calibration graph.

Under the optimized conditions, the response current of the biosensor to successive additions of glucose was recorded (**Figure 3**). A fast and sensitive response was observed after the additions of glucose. It was linear in the range of 0.1-20 mmol/L glucose with a correlation coefficient of 0.997. The biosensor reached 95% steady-state current within 15 s. The detection limit was 48 μmol/L.

The surface of the biosensor could be renewed after re-polishing on ultra fine emery paper and continued for the fabrication of biosensors. In a series of 6 independent sensors, a relative standard deviation (RSD) of 5.6% was obtained by analyzing 1 mmol/L glucose, which indicated that the fabrication procedure was reproducible. For a single sensor, a RSD obtained by 10 repeated measurements was 4.7%.

The effect of several electroactive compounds on the current response was studied. Uric acid, acetaminophen, ascorbic acid, aucrose oxalate and fructose were used as possible interfering substances. Only ascorbic acid interferes with the glucose detection since it can be oxidized directly on the electrode surface. The usage life of the biosensor was also estimated through current response. In the first two weeks, the current remained relatively constant. Then it decreased to about 80% of its initial value after one month. The composite biosensor was used to determine glucose content in commercially available beverage drinks. Sample solutions were diluted with 0.1 mol/L PBS (pH 7.0). The results obtained by using standard addition method were shown in **Table 1**.

Table 1 Recovery of glucose in real sample

Sample [a]	Original (mmol/L)	Added (mmol/L)	Founded (mmol/L)	Recovery (%)
Mixed fruit juice	0.28	0.25	0.54	104.0
Orange juice	0.32	0.30	0.61	96.7
Sport drink	0.54	0.50	1.01	94.0
Cola	0.42	0.25	0.65	92.0

[a] 1: 400 dilutions with PBS (pH 7.0).

Acknowledgments

This project was supported by Hunan Provincial Natural Science Foundation (Grant No. 04JJ30047).

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Received 4 January, 2005